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The Solution of the Structure of Spirodienone by Visual Packing Analysis

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Trial models of the rigid molecule, spirodienone, were oriented and positioned in the crystallographic cell by means of visual packing analysis. Because there are two molecules in the asymmetric unit, 12 positional parameters were adjusted independently to obtain visually 'reasonable' packing arrangements. One such arrangement refined both by varying the 12 parameters with respect to repulsion energies as well as by full-matrix group refinement with respect to observed structure factors, which are reported elsewhere. The R values of the partially refined models were 42% and 35% respectively, at which point individual atom least-squares refinement was begun yielding a final R value of 4·8%.

Introduction

The basic hypothesis of the work presented here is that there are many 'unlikely' positions for a molecule in the unit cell of the crystal lattice. Also, there are very few 'likely' positions. Therefore, by adjusting the positions and orientations of a rigid molecule in crystal space, a small number of probable or trial structures can be produced, one of which should lead to the correct structure.

The technique to be discussed employs a method of perceiving the packing environment of a molecule and then adjusting its positional and orientation parameters to improve on the packing arrangement. This method may be termed 'visual packing analysis'. A computer graphics terminal with an interactive stereo display was used to predict the packing structure of the molecule spiro[5,5]undeca-1,4,7,10-tetraene-3,9-dione or spirodienone (Fig. 1). Two separate group refinement techniques were then applied and each produced a model sufficiently near the correct structure that individual atom parameters could be refined by traditional least-squares methods: One involved packing-energy minimization using the program *PCK*5 (Williams, 1969) while the other employed least-squares refinement of rigid-group (translational and rotational) parameters (Fig. 2).

Spirodienone, $C_{11}H_8O_2$, was chosen to test the method of visual packing analysis not only because of its interesting structural, chemical, and spectroscopic properties, but also because a rigid molecule could be assumed on the basis of available chemical and structural information. However, the presence of two independent molecules in the asymmetric unit proved to be a more demanding test of the method. An instance of similar work in the recent literature is the study of GpC with data to 1·8 Å. It was refined first by energy minimization of the 'gaseous' molecule, followed by visual orientation about the postulated position of the phosphorus atom (Stellman, Hingerty, Broyde, Subramanian, Sato & Langridge, 1973).

Crystallographic data

Spirodienone was supplied by Dr Guy Farges and was crystallized from dioxane. The crystals have the space group $P\bar{1}$ with the unit-cell dimensions: $a=9{\cdot}080$ (4), $b=13{\cdot}608$ (2), $c=7{\cdot}493$ (3) Å, $\alpha=91{\cdot}11$ (2)°, $\beta=96{\cdot}91$ (4)°, $\gamma=100{\cdot}52$ (2)°. There are four molecules in the unit cell which has a calculated volume of 902·7 Å^3. The structure refined to a conventional R value of 0·048. Crystallographic and structural details are discussed elsewhere (Cullen, Hass, Klunk, Willoughby, Meyer, Farges & Dreiding, 1974).

Instrumentation

Features of the molecular display system provide that a realistic stereo image of a molecule can be produced on a mini-computer-controlled color television monitor and that the model can be manipulated by changing positional and rotational parameters. Although the full scope of the system is discussed elsewhere (Willoughby, Morimoto, Sparks & Meyer, 1974), essential here are the small size (12K word) of the computer and the advantages of ready access to a graphical display in the laboratory.

Input into the display system includes the unit-cell parameters, starting atomic coordinates, atomic connectivities, symmetry and translational operators, and a maximum of six positional and rotational parameters for the contents of the asymmetric unit. When a single, rigid molecule can be assumed per asymmetric unit, the process is straightforward. However, in the case

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of spirodienone with two molecules in the asymmetric unit, the capacity of the program was exceeded and it was necessary to use another feature of the display, namely that two separate pictures can be composed on separate portions of the disc and either displayed alternately or combined to superimpose the two separate images. Using the latter technique, each independent molecule in the asymmetric unit was manipulated, the pictures formed and overlayed, and distance calculations performed in order to obtain a quantitative indication of the intermolecular interactions.

Initial atomic coordinates

The display system requires a molecular model with which to test the packing distances. Because the structure (at this point) had not yet been solved, the coordinates of a trial model could have been obtained by converting the coordinates of a related molecule to the proper unit cell or by generating coordinates from a model-building program, *e.g.*, the program *MBLD*: *Standard Geometric Models and Cartesian Coordinates of Molecules* (Gordon & Pople, Program #QCPE-135, Quantum Chemistry Program Exchange, Bloomington, Indiana).

Because the atomic connectivities of spirodienone were already known to be rigid (Farges & Dreiding, 1966) a model with the following estimates of the different types of bond angles and distances was placed in the unit cells of both channels: C(1)–C(6) = 1·35 Å; C(1)–C(2) = 1·04 Å; C(2)–C(3) = 1·55 Å; C(3)–O = 1·00 Å; C(2)–C(1)–C(6) = 113°; C(1)–C(2)–C(3) = 118°; C–H = 1·00 Å; C(2)–C(3)–O = 115·3°; C(2)–C(3)–C(4) = 142°; C(1)–C(6)–C(5) = 119°. Hydrogen atoms were included because hydrogen interactions are critical in determining molecular interactions, both visually and in packing calculations. The two molecules together were defined as model I and visual packing analysis was initiated.

Molecular packing

In order to expedite the location of the 'correct' packing configuration, we employed the general empirical principles of crystalline packing described by Kitaigorodskii (1955) and used the graphical display to study the general features of molecular packing. During this study these principles evolved into practical guides that are useful in defining and testing trial models:

(1) In general, close packing involves fitting the 'bumps' of one molecule into the 'hollows' of neighboring molecules.

(2) Relevant to spirodienone, the space group $P\bar{1}$ contains alternating layers of molecules.

(3) No isolated polarities appear perpendicular to the molecular layers.

(4) To obtain optimum packing the molecular axes are often inclined relative to the crystal axes.

(5) Many molecular packings show a 'herringbone' configuration.

The final criterion in deciding whether a packing arrangement is a reasonable model and worthy of the time and cost of further refinement was the number and magnitude of the intermolecular contacts made with

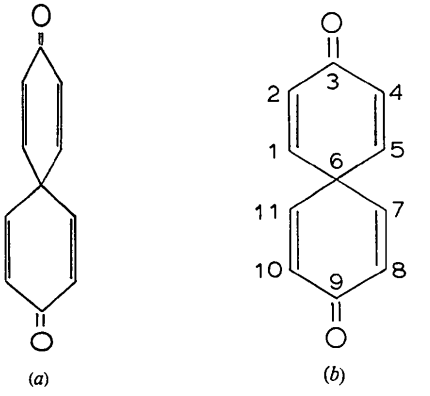

Fig. 1. (*a*) Three-dimensional representation of spirodienone. (*b*) Atomic nomenclature of spiro[5,5]undeca-1,4,7,10-tetraene-3,9-dione, spirodienone.

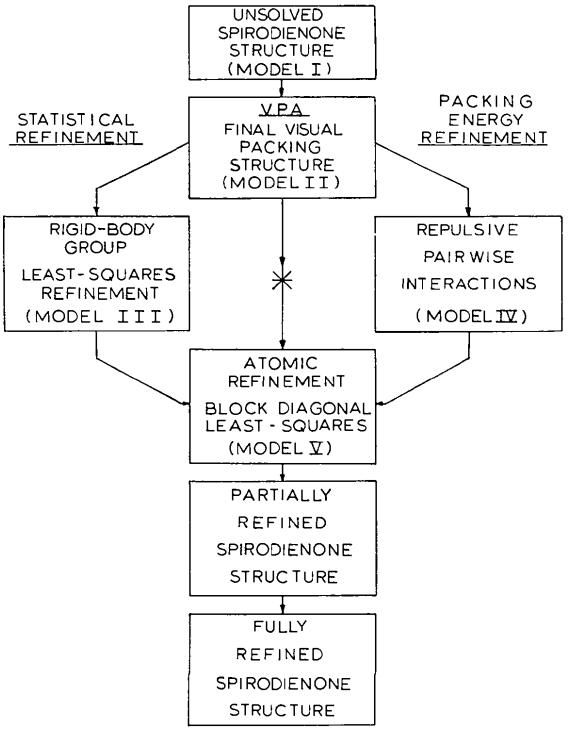

Fig. 2. Flow diagram of visual and calculated procedures used in implementing visual packing analysis to solve the spirodienone structure. (*) indicates that model II could not be refined by conventional, individual atom refinement techniques. Model V is used in a generic sense to indicate two structures, the one resulting from applying atomic refinement to the repulsive interaction model of *PCK*5 calculations (model IV) and the other one from applying atomic refinement to the results of the rigid-body group refinement calculations (model III). In both cases the atomic refinement led to models with R values of 0·13.

adjacent molecules. In general hydrogen–hydrogen distances should not be less than about 2·2 Å while all other contacts should not be less than about 3·3 Å. When such a geometry is found, the atomic coordinates are obtained from the display system and used in testing the potential refinement capability of the model. Rather than being just a 'blind search', the above procedures permit the crystallographer to learn and use spatial and intuitive powers of perception. A period of several weeks was required for one of us (B.S.H.) to generate nine candidate structures, one of which refined (see below). Owing to limited facilities for man–machine interaction on this prototype system, a certain complexity was associated with the independent adjustment of the 12 parameters.

Packing energy minimization

Although it was of great value in later refinement, the structure factor discrepancy index, R, defined by $R=\{\sum|F_o|-|F_c|/\sum|F_o|\}$ was initially of little utility because it gives a very precise summary of the correctness of each atomic position. Here, we wish to obtain an approximate position of the entire molecule near enough to the final position to permit refinement. If several atoms of the entire structure deviate significantly from the correct positions, the R value could be high, giving a false sense of incorrectness for the model as a whole. For example, of the nine trial models that were tested, the successful model (model II) had an initial R of 0·693 while other trial structures that yielded somewhat lower initial R values could not be refined further than about 50%. Least-squares refinement of the individual atomic parameters of model II was unsuccessful before rigid-body constraints were applied.

Therefore, at this point the choice of using the packing energy approach or the rigid-group refinement procedure was made (see Fig. 2). Although both of these programs required a large computer (*e.g.*, IBM 360 and CDC 6600) the more practical choice from the standpoint of computer time and test criteria was the packing program which is based on calculation of intermolecular potential energy. *PCK*5 (Williams, 1973*a*) minimizes the repulsive energy term

$$\mathrm{RP}=\tfrac{1}{2}\sum w(d_o-d_c)^2$$

where the empirical potential parameters are defined as: d_o = nonbonded interatomic distances; d_c = calculated distance for a given trial model, $d_o>d_c$; w = weighting factor; and where the sum is over all nonbonded interatomic contacts of the reference molecule with all neighboring molecules.

The values of the potentials used were those of Williams (1973*a*, *b*):

	d_o	w_p
C–C	3·65	1·87
C–H	3·03	1·56
C–O	3·40	1·87
O–O	3·40	1·87
O–H	2·90	1·56
H–H	2·88	1·00

After a likely packing arrangement was found, refinement by minimizing repulsive energy (*PCK*5) was initiated. The visually packed, trial model that successfully refined (model II) yielded a conventional R value estimate of correctness of 0·69 before *PCK*5 refinement and 0·42 after refinement. In addition, the value of the RP of model II (which serves as an estimated of goodness of fit) was 1·81. The trial model is labeled model IV after refinement by *PCK*5. The eight other visual trial models that were processed by *PCK*5 converged at RP ≥ 3·0. Later after the structure had been fully refined, the final coordinates were subjected to *PCK*5 in order to determine the 'ultimate' RP value, which turned out to be 1·4. Subsequent refinement of model IV coordinates of O and C atomic positions reduced model IV from its R value of 0·42 to 0·13. The resultant coordinates are labeled model V (see Fig. 2). Thus, the sequence: visual refinement to *PCK*5 to individual atom least-squares refinement, with the corresponding reduction of the R value from 0·69 to 0·13, shows the utility of visual packing analysis to produce trial models which can be quickly checked for convergence. Model V refined to $R=0{\cdot}048$, $Rw=0{\cdot}047$, based on F. The error in an observation of unit weight was 1·50. The final refinement and structural results are described elsewhere (Cullen *et al.*, 1975).

Rigid-group refinement

Rigid-group refinement can also serve the dual purpose of providing a test criterion (a computed R value) and an intermediate refinement procedure. This method requires a set of atomic coordinates to be defined relative to the rigid groups, which in the case of spiro-

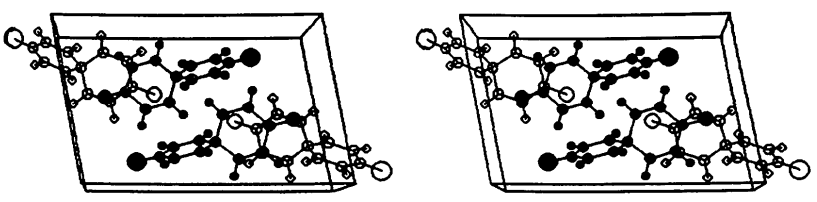

Fig. 3. Stereo view of model IV. Solid and clear circles distinguish the two molecules of the asymmetric unit.

dienone are the two molecules in the asymmetric unit. These internal coordinates are then referenced to the crystal coordinates of the model and a set of reference translational and rotational parameters obtained by *RBANG*, a program of S. F. Watkins. These parameters are in turn submitted to a full-matrix least-squares program to refine the rigid-groups' parameters. After four cycles, model II refined to $R=0{\cdot}349$ by this method (model III). The non-hydrogen atoms of model III could then be refined by the block-diagonal least-squares method which converged to model V ($R=0{\cdot}13$).

The overall angular and translational relations among the various models are given in Tables 1 and 2, while the values of the root-mean-square and maximum atomic deviation between various models are given in Table 3.

Table 1. *Translational distances* (Å) *of spiro carbon atoms between various models*

Diagonal element of the table is distance between spiro carbon of first molecule and spiro carbon of second molecule in the same model. Element of upper-right triangle is distance between spiro carbon atoms of the first molecule of the asymmetric unit for the designated models. Element of the lower-left triangle is distance between spiro carbon atoms of the second molecule of the asymmetric unit for the designated models.

Model	I	II	III	IV	V	FRS*
I	0·00	6·53	6·53	6·53	6·47	6·48
II	3·08	8·22	0·11	0·30	0·13	0·12
III	3·15	0·89	8·28	0·24	0·06	0·06
IV	3·07	0·09	0·12	7·98	0·18	0·19
V	3·06	0·12	0·12	0·06	8·12	0·01
FRS*	3·07	0·12	0·12	0·06	0·01	8·14

* FRS = fully refined structure.

Table 2. *Angles* (°) *between comparable planes of the models*

Element of upper-right triangle of the table is angle of rotation between the same rings of the first molecule of the asymmetric unit for the designated models. Element of the lower-left triangle is the angle of rotation between the comparable ring of the second molecule of the asymmetric unit for the designated models.

Model	I	II	III	IV	V	FRS
I	–	65·7	69·0	71·3	70·8	70·8
II	53·4	–	10·1	21·4	13·6	13·5
III	46·8	7·1	–	11·4	3·6	3·5
IV	38·4	15·0	8·8	–	8·2	8·3
V	44·1	10·0	2·8	6·8	–	0·1
FRS	44·1	9·9	2·8	6·8	0·1	–

Summary and conclusions

The refinement of trial structures by packing energy minimization has been previously employed (Williams, 1973*a*; Stellman *et al.*, 1973; Coiro *et al.*, 1973; Zugenmaier & Sarko, 1972). In this study the coordinates of a rigid molecule, spirodienone, placed at a random position in the unit cell, were adjusted for translation and rotation by numerical input to a laboratory mini-computer. The resultant model was displayed in three dimensions on a color television monitor. By making favorable positional adjustments, a refinement process is visually controlled.

Manifestations of several of the empirical packing guides discussed (see above) are evident in the final packing arrangement. Alternating layers of molecules generated by symmetry relations are found in all three dimensions (#2), and no polar interactions are found normal to these planes (#3). The molecules do indeed incline to the crystal axes (#4), as well as to one another to produce the predicted 'herringbone' arrangement (#5) as is shown in Fig. 3. The unique spacial arrangement of the spiro geometry as well as the fact that the graphics display does not produce a 'solid' image has made it possible to apply the 'bumps in hollows' principle (#1) only in a qualitative sense.

In using visual packing analysis to provide an initial model for the solution of crystal structures of molecules, the following points may be noted concerning the method:

(1) It was able to provide a successful model for the structure of spirodienone and although the R value of the best visual model was very high, eventual refinement was possible.

(2) Phases are obtained directly from the molecular model. Thus, visual modeling methods should work equally well for non-centrosymmetric space groups.

(3) Additional routines could permit manipulation of internal motions of the molecule (usually torsion angles) so that a rigid molecule would not be prerequisite. These routines are under development.

(4) With practice one can learn to make more judicious changes in the parameters to avoid poorer models in favor of more likely ones.

(5) An expanded mini-computer system will permit future versions of visual packing analysis in our laboratory to have *PCK5* linked directly to the display program. Then the sequence of events could flow

Table 3. *Values of the root-mean-square and the maximum change in atomic positions between various models*

The upper triangle gives the root-mean-square (RMS) shifts in Å of carbon and oxygen atoms. The lower triangle lists the atoms that possess the largest deviations. ΔD in position as the atom changes position from one model to another. ΔD is given in parentheses in Å.

		RMS			
Model	I	II	III	IV	FRS
I	–	0·455	–	–	0·436
II	O(1) (0·780)	–	0·319	0·068	0·126
III	–	C(10) (0·286)	–	–	0·159
IV	–	O(2) (0·119)	–	–	0·101
FRS	O(1) (0·800)	C(4) (0·248)	C(11) (0·238)	C(4) (0·222)	–

smoothly from visual models to packing refinement to 'before and after' views, all within a few minutes. This would not only lead to quicker model evaluation, but would also help avoid the occasional problem of false minima that can arise when *PCK5* is used blindly.

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The Crystal and Molecular Structure of α Heteronium Bromide

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The crystal and molecular structure of 3-hydroxy-1,1-dimethylpyrrolidinium bromide α-phenyl-2-thiopheneglycolate (α heteronium bromide) $C_{18}H_{22}NO_3SBr$, was determined by single-crystal X-ray diffraction techniques. The crystals were orthorhombic, $D_m = 1{\cdot}5$ g cm^{-3}, space group $Pna2_1$, $Z=4$, $a=18{\cdot}596$ (1), $b=8{\cdot}049$ (9), and $c=12{\cdot}297$ (8) Å. The structure was solved using 1671 observed independent reflections to yield a final R value of 0·044.

Introduction

The crystal structure of the anticholinergic drug 3-hydroxy-1,1-dimethylpyrrolidinium bromide α-phenyl-2-thiopheneglycolate (heteronium bromide) has been solved. Heteronium bromide inhibits the muscarinic action of acetylcholine and thus suppresses secretory processes as well as other physiological functions (Chernish & Rosenak, 1965; Shay & Komarov, 1945). Heteronium bromide, therefore, has been therapeutically employed to reduce acid secretion in animals with duodenal ulcers (Shay & Komarov, 1945), and as a general antispasmodic agent (Ryan & Ainsworth, 1962).

The ability of heteronium bromide to elicit the responses is presumably due to its structural similarity with that portion of the acetylcholine molecule which bonds at the muscarinic receptor site. Many X-ray structural studies have been conducted in an attempt to define the three-dimensional structure of the receptor sites involved in these events (*e.g.* Meyerhöffer, 1970; Meyerhöffer & Carlström, 1969). The specific stereochemistry of the receptor site will require further investigation of the resolved isomeric compounds which function as anticholinergic agents.

Experimental

Heteronium bromide was obtained from the Eli Lilly Drug Co. as a powdered racemic compound prepared by the method of Ryan & Ainsworth (1962). The α and β diastereoisomers were resolved by fractional crystallization from methanol/ethyl acetate (1:4 v/v) to yield the pure α isomer with a m.p. of 208–211 °C. Analysis of the crystalline α isomer yielded C = 52·39%, H = 5·33%, compared to the theoretical C = 52·43% and H = 5·38%. The crystals used for the X-ray analysis were grown by the vapor-diffusion method using a methanolic solution of the compound and ethyl acetate as the vapor phase. The approximate unit-cell dimensions and the space group were determined from photographs with Mo $K\alpha$ radiation. The density was obtained by the flotation method using a mixed solvent of tetrabromoethane and ether.

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